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PROCEEDINGS

Mechanism of Ennoblement by Biofilms on Active/Passive Alloys Immersed in Seawater

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Introduction

The purpose of this paper is to present a brief review of how microbial fouling films affect the corrosion behavior of passive metals in natural waters. It has often been reported that microbial films shift the open circuit corrosion potential (OCP) of passive metals in the positive (or noble) direction and enhance the kinetics of the cathodic oxygen reduction reaction[1-10]. The significance of this effect lies in it's influence on localized corrosion initiation and propagation. In chloride bearing waters, initiation of pitting and crevice corrosion is statistical, with the probability of initiation increasing directly with chloride ion activity and OCP. Thus, at a given chloride level, the probability of localized corrosion initiation is increased by anything (such as a biofilm and its metabolic products) that causes the OCP to become ennobled.

The investigators cited above[1-10] found that the OCP of an alloy resistant to chloride initiation of localized corrosion, such as N08367 (6XN), usually became shifted to a steady noble value between +350 and +500 mV vs. the saturated calomel electrode (SCE) as a microbial biofilm formed at the metal/water interface[9]. In contrast, the OCP of the same metal in the same water ranged from -100 to +150 mV SCE if the microbial biofilm was inactivated or prevented from forming[9]. On alloys with less resistance to localized corrosion (e.g., S30400), the OCP was first shifted noble, but as soon as pitting or crevice corrosion initiated, the current supplied by the active corrosion polarized the potential back to a

more active value[9]. Thus, potential ennoblement is only sustained for long periods of time in the absence of corrosion initiation.

It has been established by many authors, as reviewed by Scotto[7] and Dexter[10] that ennoblement of the OCP in the presence of a microbial film is a direct result of the metabolic activity of microorganisms in the film. Other citations in the literature report no ennoblement effect. Mansfeld et al.[11] showed that ennoblement did not occur under conditions in which the film consisted of a scatter of individual cells (insufficient surface coverage), while Little, et al.[12] showed no ennoblement when a very thick film formed rapidly. Thus, the question arises as to what factors promote or suppress the effect. Biofilms formed from natural aqueous environments are noted for providing spotty, rather than continuous coverage of solid surfaces. Dexter, et al.[13] showed that 30 to 40 percent coverage was required for the OCP to rise above the +150 mV SCE level, while substantially complete coverage was required for the maximum amount of ennoblement.

Dexter and Zhang[8,9] have shown that biofilms grown at all salinities from 0.02 to 28 ppt were able to ennoble the OCP. The most noble potentials (about +500 mV SCE) and the largest amount of ennoblement (over 500 mV) were found in fresh water, and both decreased with increasing salinity[8,9]. Temperature in the range of 2 to 30°C and dissolved oxygen content in the bulk water from 0.5 to 10 ppm have small effects in the direction predicted by electrochemical thermodynamics[14].

ENNOBLEMENT MECHANISMS

Biofilms can affect electrochemical parameters at a metal surface through both their physical presence and their metabolic activity. Physically, the film acts as a diffusion barrier, tending to concentrate chemical species produced at the metal-film interface and to retard diffusion of species from the bulk water toward the metal surface. One effect of reduced diffusivities[15] perpendicular to the metal surface is to concentrate acidic by products from the metabolism of microorganisms in the biofilm. It is well known that the reversible oxygen potential shifts about 60 mV in the noble direction for each unit decrease in pH. This more than balances the decrease in potential as dissolved oxygen is decreased by biological processes[13]. Based on thermodynamics, the maximum calculated ennoblement[13] for a reversible oxygen electrode

would be 280 mV for changes in pH and O_2 from 8 (and 0.2 atm) to 3 (and 0.02). Since the oxygen electrode is not strictly reversible, even on platinum, one would expect the actual noble shift to be smaller than the calculated value. Actual measurements have shown that the OCP for a pH change from 8 to 3 in both aerated and deaerated seawaters varied from 170 mV on platinum to nearly 250 mV on S31600 stainless steel[13,16]. These data show that the often observed ennoblements of 250 mV or less on common stainless steels in natural seawater can be explained by the pH mechanism alone for a pH of about 3 at the metal surface. The Larger amounts of ennoblement often observed on superalloys and platinum in seawater and on all alloys in lower salinity waters, however, require another mechanism in addition to that of pH.

One question is whether a pH of 3 is possible at the metal surface under a biofilm. We have two types of evidence that it is. In one set of experiments, organisms scraped from marine biofilms on platinum were grown on Difco Marine Agar containing various pH indicating dyes. The pH of many of the colonies that grew was observed[17] to be below 3. In other experiments currently under way we are measuring the pH directly using microelectrodes of the iridium oxide type (Montana St. University). These data show variations from point to point along the metal surface with pH values ranging from 0.4 to 3.7.

Scotto proposed that oxygen reduction at the cathode surface might be enhanced by bacterially produced enzymes[17]. One possibility for this mechanism[13] involves the suite of enzymes that bacteria produce to control toxic oxygen derivatives. Superoxide, hydrogen peroxide, and hydroxyl radical are all toxic by-products of oxygen reduction (or respiration). The concentration of these by-products must be controlled by the organisms for survival. The enzyme, superoxide dismutase, catalyses the destruction of superoxide free radical (O_2^-) by Equn (1) below. Superoxide dismutase, however, is inactivated by the build up of H_2O_2. Therefore, it is associated with two other enzymes, catalase and peroxidase, which break down H_2O_2 according to Equn (2):

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \qquad (1)$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad (2)$$

We have proposed[13,17] that the mechanism for ennoblement may involve both low pH and peroxide within the biofilm. Peroxide would contribute to the mechanism by virtue of its noble redox potential (1.76 V NHE vs. 1.23 V for oxygen). The toleration limit of most microorganisms for H_2O_2 is about 15 mM[18]. The above reactions assure that some peroxide will always be present within the biofilm, but limit the concentration. Peroxide in the high micromolar to low millimolar range has been identified[17] in the film by peroxide indicating enzyme strips (from E. Merck Co.). Chemical simulation experiments on bare platinum electrodes have verified that additions of peroxide in this range shift the OCP of platinum in the noble direction. The only set of chemical conditions we found[13] that was able to reproduce the OCP of +400 to 450 mV SCE observed for biofilmed platinum was deaerated seawater with less than 0.5 ppm O_2 and 250 μM peroxide at pH 2.9. Cyclic voltammetry also reproduced the peroxide peak from the biofilmed platinum electrode only under this same set of conditions[13]. It is significant to note that, although the oxygen concentration at the metal surface must be low, the entire effect disappears if the bulk environment is made anaerobic[19]. This means that some oxygen must reach the metal surface, but considerable variability from point to point along the surface is also possible.

Another suggested mechanism for ennoblement involves the catalytic action of bacterially produced organo-metallics on the oxygen reaction[2,5]. Such compounds are used to catalyze reduction of oxygen to water in fuel cells[20]. A transition metal ion (such as Fe, Mn, Co or Ni) is chelated in a square planar complex by a macrocyclic organic molecule of the phthalocyanin, porphyrin or tetraazaannulene types, having four nitrogen atoms as ligands[20,21]. The enzymes mentioned above are also porphyrin based organo-metallic complexes. Thus, we now believe that this mechanism is essentially the same as the enzyme mechanism, and that both of them involve hydrogen peroxide and heavy metals. Most of the passive alloys ennobled by biofilms contain one or more of the necessary heavy metals as alloying elements. Another source of heavy metals is the seawater itself. Microorganisms in the biofilm require most of these heavy metals as micronutrients. Hence, in a mature biofilm the needed heavy metals, as well as the porphyrins, may be provided by decaying organisms at the base of the film. EDAX data on biofilms grown on platinum at our location showed Fe and Mn peaks[19].

Based on the literature and research results outlined above, the following general model for the ennoblement mechanism is proposed and illustrated schematically in Figure 1. A shift in the OCP to values in the +300 to +500 mV SCE range by biofilms less than about 100 μm thick requires sharp gradients in both pH and dissolved oxygen within the film. The outer layers of the film should be aerobic and at near neutral pH, while immediately adjacent to the metal surface the film will be acidified, with a generally low oxygen concentration, possibly becoming anaerobic over some portions of the metal surface. Under these conditions the primary contribution to ennoblement would come from the thermodynamic effect of pH on the OCP. An important secondary contribution would come from the cathodic reaction: the acidic form of O_2 reduction (when O_2 is present, Equn. 3) and the reduction of H_2O_2 to water (Equn. 4).

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \qquad E^\circ = 1.229 \text{ V SHE} \qquad (3)$$

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \qquad E^\circ = 1.776 \text{ V SHE} \qquad (4)$$

Both of these reactions have more noble redox potentials than the oxygen reaction at neutral pH. The metabolic action of the biofilm itself would be both the primary source and regulator of peroxide through the enzyme system of the aerobic organisms as described above. We also think that peroxide, being a powerful oxidizer may help in maintaining the low pH within the film through the oxidation of reduced heavy metals, which could then hydrolyze to form more acid within the film. This suite of conditions would require consortia of various types of microorganisms as typically found in biofilms grown from natural waters. The outer portions of the film would contain mostly aerobic and acid producing organisms consuming oxygen. Beneath these would be the Fe and Mn reducers and the fermenters. Finally, at the base of the film, the anaerobic sulfate reducing bacteria (SRB) may play a role.

This general biofilm model is supported by a vast literature on bio-geochemical cycling of heavy metals in marine sediments and aquatic environments[22-25]. The material in citations 22 through 25 give a picture of the cycling of Fe and Mn in environments with limited oxygen availability, such as we find in mature biofilms. The main difference is in the physical dimension over which the cycling takes place. As illustrated schematically in Figure 2, this cycling in stratified lakes takes place over

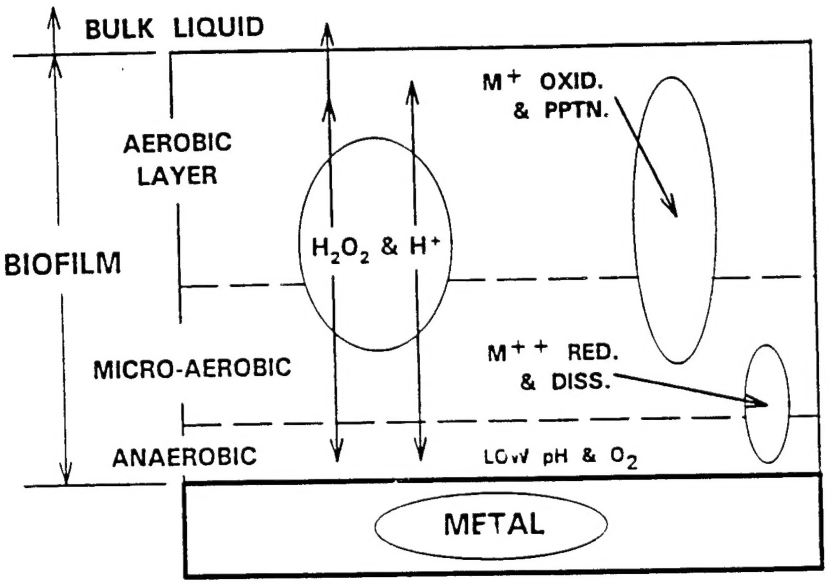

Figure 1. Schematic diagram of the biofilm model showing production and diffusion of H_2O_2 and acidity. Heavy metal cycling takes place by both chemical and biological processes.

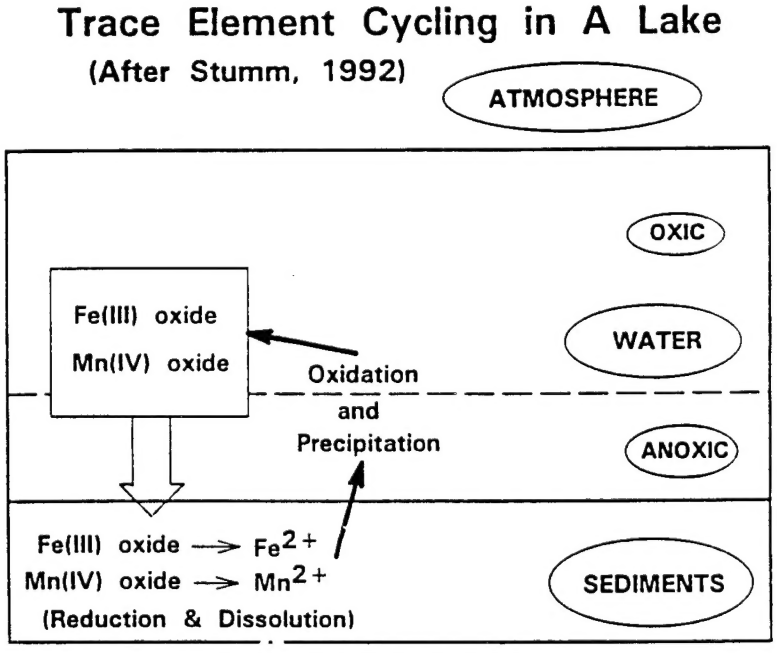

Figure 2. Heavy metal cycling by chemical and biological processes in a stratified lake. (Redrawn from Stumm[22]). Note the similarity to the biofilm processes in Figure 1.

dimensions of meters[22]. In marine sediments, the same thing takes place within centimeters, while within a biofilm it occurs within about 100 μm.

One or more species of SRB are known to be dominant in many forms of MIC. Do they play a substantial role in the ennoblement process? They are certainly present in the coastal and estuarine waters that produce ennoblement at the Lower Delaware Bay site. So far, we think that they are not important in maintaining ennoblement under a mature biofilm. This judgment is based on four types of data. First, the pH is generally too low in the anaerobic portions of the film near the metal surface, although the model would accommodate neutral pH micro-niches in which they might flourish. Second, they cannot be dominant because ennoblement is lost if one makes the entire environment anaerobic[19]. The third and fourth types of data have been obtained in our lab just recently, and they will be published in detail elsewhere. Radioisotopic studies have been done to determine if appreciable sulfate reduction is taking place in the biofilm. Ennobled platinum coupons were incubated in seawater containing the radioactive tracer $^{35}SO_4^{-2}$ as the sodium salt. When SRB are active, they convert this to $H_2{}^{35}S$, which is trapped and then detected by a scintillation counter. Large signals were obtained when this technique was used on samples of anaerobic sediment containing SRB. In contrast, no detectable signal was produced by the organisms in the ennobling biofilm. Finally, ennoblement on platinum was neither reduced nor eliminated by exposing the coupons to seawater containing the SRB inhibitor, molybdate.

This model accomodates two major points that have been brought up in discussions of the mechanism. Some argue that oxygen cannot be involved because microelectrode measurements have shown zero oxygen tension at the metal surface under a mature biofilm[26]. The model allows for large portions of the metal surface to be anaerobic as long as it is not totally so. Others contend that bacterial enzymes are active only at neutral pH values[27], so ennoblement by that mechanism cannot occur at acid pH. The model as proposed requires only the inner portions of the film to be acidified.

EFFECT ON CORROSION

The question now is, what effect does the observed ennoblement of the OCP have on corrosion of passive metals and alloys in marine

environments? Initiation of pits on passive alloy surfaces in chloride containing media depends on chloride ion concentration and electrode potential. The more electropositive (or noble) the potential, the easier it will be for chloride ions to penetrate the passive film and create small anodic areas. Thus, anything that causes the OCP to change in the positive direction (such as ennoblement by biofilms) increases the probability of pit initiation at a given chloride ion concentration. If one could measure the critical pitting potential at the metal-biofilm interface, it would be possible to predict when a biofilm would cause pit initiation just by measuring the OCP. Dexter and Zhang[8,9] tried comparing maximum ennobled potentials to the critical pitting potential for stainless alloys S30400 and S31600 as a function of water salinity. From this data they concluded that the maximum salinities at which these alloys should be resistant to pit initiation in the absence of crevices should be reduced by the ennobling action of a biofilm. The general conclusion is correct, but the results cannot be used for predicting localized corrosion behavior in practical applications for at least two reasons. First is the uncertainty in chemistry at the metal surface under a biofilm[13], and second is that crevice corrosion occurs at lower potentials than pitting.

Under service conditions, crevices are always present. Johnsen and Bardal said that OCP ennoblement should decrease crevice initiation times and increase propagation[2,3]. Dexter et al. showed that bacteria in the crevice solution could contribute to depletion of oxygen, potentially decreasing crevice initiation times[28]. Other tests of crevice initiation times in our laboratory have sometimes shown a decrease in crevice initiation times by factors of 2 to 5, but the results are not consistent[29]. The data are much more convincing for the effect of biofilms on crevice propagation. Recently, Zhang and Dexter have used remote crevice assembly tests to show that propagation of crevice corrosion is accelerated by biofilms on alloys with low resistance (S30400 and S31603) and intermediate resistance (N08904 and S31725) to crevice corrosion in coastal seawater[29]. Crevice corrosion current densities (see Figure 3) were one to three orders of magnitude higher in natural seawater than in a control water made by filtering to remove most of the film forming microorganisms. On these same alloys they also measured increases in: percentage of crevice area attacked under the washer, maximum and average depths of attack, and weight loss for the biofilmed samples versus controls[29]. Gallagher, et al.[6] found similar results. Zhang and Dexter concluded that acceleration of crevice corrosion by the action

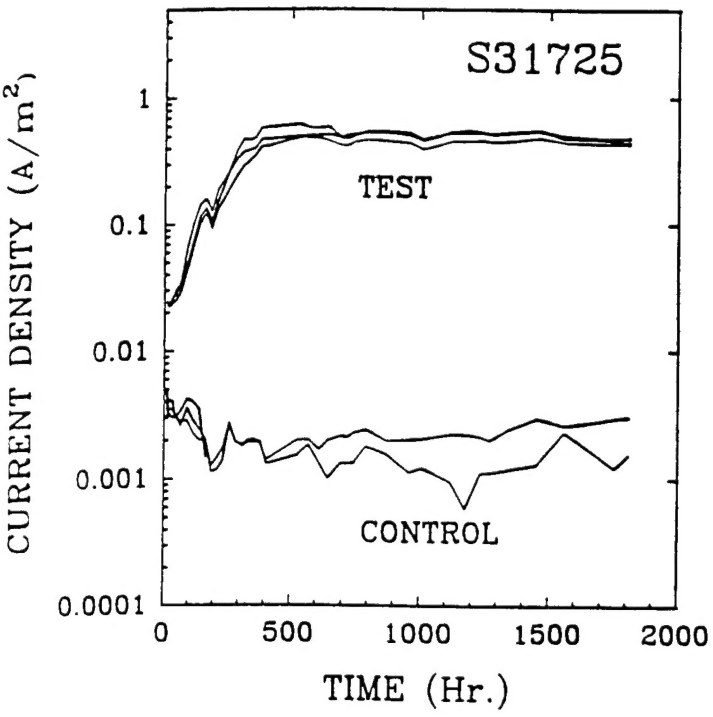

Figure 3. Crevice corrosion current density vs. time from remote crevice assembly tests on biofilmed (test) and bare (control) samples of alloy S31725 as read on a zero resistance ammeter (after Zhang and Dexter[29]).

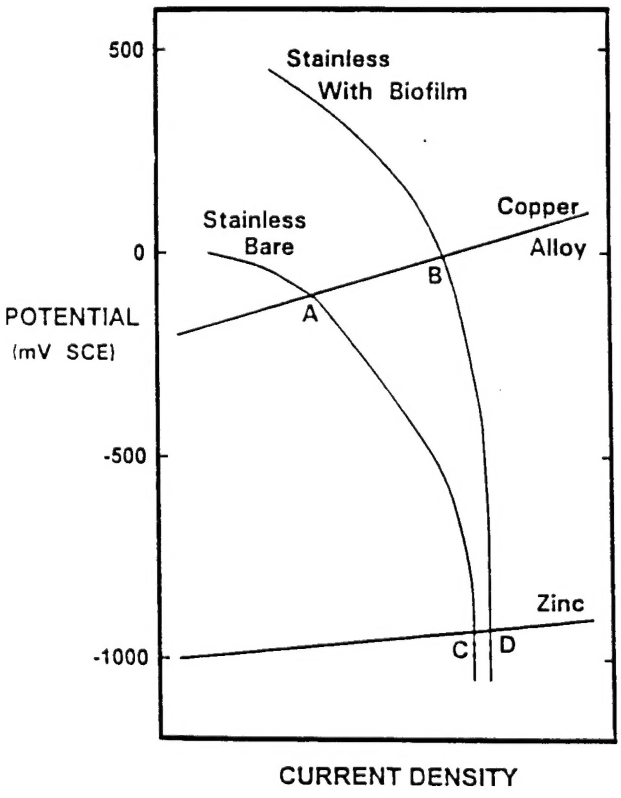

Figure 4. Schematic polarization diagram showing the influence of a biofilm on the stainless steel cathode of galvanic couples with copper and zinc. A greater effect of the biofilm on galvanic corrosion is predicted for copper than for zinc.

of biofilms in their coastal waters was more significant on the intermediate resistance alloys, N08904 and S31703, than it was on S30400 and S31603, which corrode readily with or without biofilms. The biofilm had no effect in their two-month tests on alloy N08367.

The rate of galvanic corrosion is often controlled by the reduction reaction on the cathodic member of the couple. Therefore, any increase in kinetics of the cathodic reaction on passive metals has the potential to increase the severity of these metals as cathodes in galvanic couples[13]. Work summarized by Scotto[7] showed that the upper portion of the cathodic polarization curve was shifted toward higher current densities, while the lower portion, dominated by the limiting current for oxygen diffusion, was affected to a lesser extent. These data suggested that the rate of galvanic corrosion on the anodic member of the couple should be affected by the action of a biofilm more (as shown in Figure 4) if the mixed potential of the couple is within the upper portion of the cathodic polarization curve than if the mixed potential is more negative. For example, if copper and steel were both connected to similar biofilmed stainless steel cathodes, the data predict that corrosion of copper will be accelerated more by the action of the biofilm than corrosion of steel. An important related question is whether consumption of Al, Mg and Zn sacrificial anodes is increased by the action of biofilms on the protected structure. The cathodic polarization data predict the effect on these active materials will be small because the couple potential is very negative.

Galvanic corrosion experiments in coastal seawater have been run for 24 Hr on couples of copper, 90-10 Cu-Ni, 1020 steel, 3003 Al and Zn, each connected to bare (control) and ennobled passive alloy cathodes, and the galvanic corrosion currents were read on a zero resistance ammeter. The results for steel and aluminum showed that the galvanic current for the couple with the filmed cathode was nearly an order of magnitude larger than that with the bare cathode[13]. The data for Zn showed the reverse effect[13], the current for the couple with the filmed cathode being less than control by a factor of 2. These data are consistent with the idea that biofilms on the cathode increase the rate of galvanic corrosion. Coupling to an active anode such as Zn, however, produces enough alkali at the metal surface to interfere with the metabolism of microorganisms in the biofilm[30]. Instead of stimulating the cathode reaction in this case, the biofilm acts as a diffusion barrier. Experiments are currently underway to obtain longer term data.

SUMMARY

The mechanism of ennoblement is not yet completely understood. A model has been proposed in which the effect is explained by a decrease in pH and an increase in hydrogen peroxide concentration at the metal surface under the biofilm. The model calls for a layered biofilm structure with sharp gradients in both oxygen and pH. The outer layers of the film are at ambient pH and oxygen concentration, while the inner layers are acidic with low oxygen. Within the film, acids are produced by the consortia of organisms, and peroxide is both produced and regulated below toxic levels by bacterial enzymes. Acidity and peroxide produced in the film diffuse both toward the metal and the bulk liquid, building up in the low oxygen environment near the metal surface, but being neutralized in the outer portions of the film. Accumulation and hydrolysis of heavy metals help establish the low pH. The model is consistent with bio-geochemical data showing heavy metal cycling in stratified lakes and marine sediments.

Ennoblement of the OCP increases the propagation rate of crevice corrosion on susceptible passive alloys, and it may also decrease the initiation time. Short term (24 hour) tests showed that biofilms can increase the severity of stainless steels as the cathodes of galvanic couples when the anode member of the couple is more positive than Al. For very active anodes, such as Zn, the biofilm acted as a diffusion barrier, reducing the galvanic corrosion rate. Predictive capability for the effect of biofilms on localized corrosion awaits a greater understanding of the chemistry at the metal surface and its variability under the biofilm.

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